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[Role of Nitric Oxide in a Model of Toxic Exposure]

Mark L. Witten, Ph.D.

The University of Arizona, Tucson, AZ 85724

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AIR FORCE RESEARCH LABORATORY
AF OFFICE OF SCIENTIFIC RESEARCH (AFOSR)/RSA
ARLINGTON, VIRGINIA 22203
AIR FORCE MATERIEL COMMAND

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14. ABSTRACT
We have demonstrated over the past three years that JP-8 and S-8 jet fuels have different sites of primary injury in the lungs. Additionally, we have determined that S-8 jet fuel generates a higher nitric oxide response than JP-8 jet fuel. These data have major implications for the U.S. Armed Forces as synthetic fuels development increases to replace oil sources.

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AFOSR Final Report

(1) Abstract

We have spent the last three years elucidating the role of nitric oxide production and sites of lung injury in the lungs due to JP-8 jet fuel and S-8 jet fuel exposure. We have found that S-8 jet fuel exposure produced higher amounts of nitric oxide production. Additionally, the site of initial lung injury for JP-8 jet fuel exposure was the type II alveolar epithelial cells while S-8 jet fuel exposure caused injury at the terminal bronchioles.

(2) Objectives

(a) To determine the role of nitric oxide in initiating the lung injury cascade as demonstrated by alveolar epithelial type II cell exposure.

(b) Compare the sites of initial injury in the lungs between JP-8 and S-8 jet fuels.

(c) Pulmonary evaluation of S-8 jet fuel exposures.

(3) Findings

Objective A- We studied the effect(s) of JP-8 and S-8 jet fuel exposure on cultured rat alveolar epithelial type II cells with respect to cell viability and nitric oxide synthesis. The jet fuel exposure times varied from 0.25, 0.5, 1.0, and 6.0 hours at the following jet fuel exposure concentrations; 0.0, 0.1, 0.4, and 2.0 micrograms/ml. These exposure concentrations correlate to 0, 100 mg/cubic meter, 400 mg/cubic meter, and 2000 mg/cubic meter jet fuel concentrations in a military setting.

The data demonstrated that JP-8 jet fuel exposure presents a gradual decline in cell viability and steady elevation in nitric oxide release as JP-8 concentrations increase. At the 2.0 microgram/ml JP-8 jet fuel concentration level, nearly all of the rate alveolar epithelial type II cells were dead. Moreover, S-8 jet fuel exposure to the rat alveolar epithelial type II cells demonstrated an abrupt fall in cell viability and increases in nitric oxide concentration, particularly at the 2.0 microgram/ml exposure level at one and six hours exposure time periods. At 0.0, 0.2, and 0.4 micrograms/ml S-8 jet fuel concentrations, the cell viability levels were constant. These data suggest that JP-8 and S-8 jet fuels cause different alveolar epithelial nitric oxide and toxicity (% cell viability) responses.

Objective B- This study was designed to characterize and compare the pulmonary effects in the distal lungs from a low level exposure to JP-8 and S-8 jet fuels. We hypothesize that both fuels have different airway epithelial deposition and toxicity responses. Male C57BL/6 mice were exposed to S-8 and JP-8 jet fuels at an average concentration of 53 mg/cubic

meters for one hour/day for seven days. A pulmonary function test performed at 24 hours after the final jet fuel exposure indicated that there was a significant increase in both inspiratory and expiratory lung resistance compared to control mouse pulmonary function values. There were no significant S-8 or JP-8 respiratory permeability changes observed compared to mouse control values. This finding suggests that there was no significant loss of barrier permeability in these mice. Morphological and morphometric analyses of airway tissue demonstrated that both fuels showed different patterns of targeted epithelial cells, bronchioles with S-8 jet fuel exposure and alveoli/terminal bronchioles with JP-8 jet fuel exposure. These data suggest that both jet fuels, JP-8 and S-8, have partially different deposition patterns, which may possibly contribute to specific different adverse effects in lung ventilator function.

Objective C- No current studies have systematically examined the pulmonary health effects of S-8 jet fuel exposure. In order to gain an understanding about the threshold concentration in which lung injury would be observed, C57BL/6 mice were nose-only exposed to S-8 jet fuel for one hour/day for seven days at average concentrations of 0.0, 93, 352, and 616 mg/cubic meters. Evaluation of pulmonary function, airway epithelial permeability, and pathohistology was performed 24 hours after the last S-8 jet fuel exposure. We found significant differences in expiratory lung resistance and total lung compliance in the 352 mg/cubic meters exposure group, for which no clear S-8 jet fuel concentrations alterations could be determined. No significant changes in respiratory permeability were exhibited, indicating that there was no loss of epithelial barrier integrity following S-8 jet fuel exposures. However, morphological examination and morphometric analyses of distal lung tissue, using transmission electron microscopy, revealed cellular damage in alveolar type II cells, with significant increases in volume density of lamellar bodies/vacuoles at the 352 and 626 mg/cubic meters S-8 jet fuel concentration levels. Moreover, terminal bronchiolar Clara cell injury, as demonstrated by apical membrane blebs, was observed at relatively low concentrations of S-8 jet fuel exposure, 93 mg/cubic meters exposure level. This finding suggests that if S-8 jet fuel is ever utilized in military settings, the current exposure limit of 350 mg/cubic meters may require revision.

(4) Collaborations

We collaborated with many other AFOSR-funded jet fuel scientists to produce the book entitled, Jet Fuel Toxicology. This book was published on August 1, 2010. This book has been used as a reference source for chronic hydrocarbon exposure settings with respect to ongoing research at national laboratories for the Gulf of Mexico oil spill crisis.

(5) Supported Personnel

(a)	Mark L. Witten, Ph.D.	Principal Investigator	100% effort
(b)	Simon Wong, M.D.	Co-Investigator	25% effort
(c)	Cindy Fastje	Research Technician	50% effort

(6) Publications

(a) Witten ML, Zeiger E, Ritchie G, eds. Jet Fuel Toxicology.

Taylor & Francis Publishers, New York, New York. August 2010.

(b) Wong SS, Vargas J, Thomas A, Heys J, McLaughlin M, Camponovo R, Lantz RC, Witten ML: In vivo comparison of epithelial responses for S-8 versus JP-8 jet fuels below permissible exposure limit. TOXICOLOGY, 2008, 254:106-111.

(c) Harris DT, Sakiestewa D, Titone D, He X, Hyde J, Witten M:

JP-8 jet fuel exposure suppresses the immune response to viral infections.

TOXICOLOGY & INDUSTRIAL HEALTH, 2008, 24:209-216.

(d) Wong SS, Desmaris T, Lantz RC, Thomas A, Witten ML: Pulmonary evaluation of permissible exposure limit of S-8 synthetic jet fuel in mice. TOXICOLOGICAL SCIENCES, 2009, 109:312-320.

(e) Robb TR, Rogers M, Wong SS, Witten ML: The cytotoxic response to JP-8 versus S-8 jet fuel exposure in cultured rat alveolar epithelial type II cells. TOXICOLOGY & INDUSTRIAL HEALTH, 2010, 26:364-374.

(7) Patents.

PCT patent application filed on August 4, 2010 on jet fuel biosensor.

(8) Interactions

(a) Communication with British Aerospace & Engineering and Thales on commercialization of biosensor. Additionally, I am in negotiations with Ronald Michael, CEO of The Stratford Group, Inc., to purchase an equity stake in Phoenix Biometrics, Inc.